Characterization of mechanosensitive splanchnic nerve afferent fibers innervating the rat stomach

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Ozaki, Noriyuki, and G. F. Gebhart. Characterization of mechanosensitive splanchnic nerve afferent fibers innervating the rat stomach. Am J Physiol Gastrointest Liver Physiol 281: G1449–G1459, 2001.—Splanchnic nerve fibers innervating the stomach were studied in anesthetized rats; 997 fibers in the T9 or T10 dorsal roots were identified by electrical stimulation of the splanchnic nerve. Thirty-one fibers responded to gastric distension. Extrapolated response thresholds ranged between 0 and 53 mmHg; seven fibers had thresholds for response ≥50 mmHg. Thermo- and/or chemosensitivity was tested in 18 of the 31 fibers. Four of twelve fibers responded to intragastric perfusion of heated saline; none of eight fibers tested responded to perfusion of cold saline. Infusion of glucose, L-arginine, or potassium oleate produced no change in resting activity. Intragastric instillation of 12% glycerol or an inflammatory soup (bradykinin 10^{-5} M, PGE_2 10^{-5} M, serotonin 10^{-5} M, histamine 10^{-5} M, and KCl 10^{-3} M) and prior heat stimulation sensitized responses to distension. The results reveal the presence of low- and high-threshold mechanosensitive fibers in the splanchnic innervation of the stomach. These fibers have the ability to sensitize, and they likely contribute to pain and altered sensations that can arise from the stomach. visceral pain; sensitization; thermosensitivity; chemosensitivity; functional gastrointestinal disorders

AFFERENT (sensory) nerve fibers in the vagus or splanchnic nerves that innervate the upper gastrointestinal tract provide information to the central nervous system that leads to a variety of consciously perceived sensations. These sensations include satiety, nausea, bloating, discomfort, and pain, the latter associated with mechanoreceptor endings in muscle that respond to stretch or distension of the organ (see Refs. 11 and 64 for review).

The presence of populations of low- and high-threshold mechanosensitive afferent fibers that innervate hollow viscera has been well documented (7, 10, 13, 27–29, 34, 60–63, 68). Low thresholds for response are interpreted as indicating a role in regulatory functions (e.g., storage, propulsion, emptying) in addition to conscious sensations associated with nonpainful mechanical stimulation (e.g., fullness, bloating, nausea) (12). High thresholds for response have been taken as evidence for the presence of nociceptors that give rise to discomfort and acute pain (11, 64, 65). Previous studies have not identified high-threshold mechanosensitive afferent fibers in the vagus nerve (3, 16, 17, 36, 50, 51), thus indirectly supporting clinical evidence (5, 75) and the generally held notion that mechanosensitive gastric vagal afferent fibers are not important to acute stomach pain.

Visceral receptors have been classified anatomically on the basis of their location (mucosa, muscle, serosa) or physiologically on the basis of the stimuli to which they respond (mechanical, thermal, chemical), although many respond to more than one stimulus (for review, see Ref. 64). Much of the recent literature has focused on the mechanosensitivity of gastrointestinal receptors (7, 32, 39, 50, 51, 62, 63) and their potential role in visceral nociception. To our knowledge, no such study of gastric splanchnic afferent fibers has been reported. The principal aim of the present study was thus to characterize the mechanosensitivity of gastric splanchnic afferent fibers. Because mechanical distension of the stomach was achieved by fluid distension, we were also able to examine responses of mechanosensitive fibers to some chemical stimuli and to thermal stimuli. Splanchnic afferent fibers innervating the stomach have their cell bodies located in lower thoracic and upper lumbar spinal ganglia (T4–L2) in the rat (46, 67). However, the distributions of cell bodies from the three principal divisions of the stomach (fundus, corpus, and pyloric antrum) are not known, and we undertook a preliminary investigation of this question before starting the electrophysiological phase of the study. Some of these data have been presented previously in abstract form (49).

METHODS
All experiments were approved by the University of Iowa Institutional Animal Care and Use Committee.

Retrograde Labeling
The origin of the primary afferent innervation of the stomach was examined by retrograde tracing using the fluorescent dye fluorogold (FG; Fluorochrome, Denver, CO). Experiments were performed on six male Sprague-Dawley rats (400–500 g; Harlan, Indianapolis, IN). Food, but not water,...
was withheld for 24 h before surgery. The animals were anesthetized with an intraperitoneal injection of pentobarbital sodium (Nembutal, 45–50 mg/kg; Abbott Laboratories, Abbott Park, IL). The stomach was exposed by midline abdominal incision, and 2.5-μl injections of a 4% (wt/vol) suspension of FG in saline were made in the ventral (8 sites) and dorsal (8 sites) walls of the fundus, corpus, or pyloric antrum of the stomach with a Hamilton microsyringe. The needle was advanced for a distance of 0.5–1 cm from the point of insertion and was left in place for up to 1 min after injection to prevent leakage of the dye along the needle track. Immediately after withdrawal of the needle, the insertion hole was sealed with cyanoacrylate (Borden, Columbus, OH). The stomach was then thoroughly washed and swabbed with saline before the abdomen was closed.

Ten to sixteen days after FG injection, rats were deeply anesthetized with an overdose of pentobarbital sodium and perfused via the aorta with saline followed by ice-cold 4% paraformaldehyde in 0.1 M PBS. Dorsal root ganglia (DRG) were removed from rats and postfixed for a further hour in 4% paraformaldehyde at 4°C. Tissue was then washed extensively with 20% sucrose phosphate buffer for at least 24 h before use.

DRGs were sectioned on a cryostat at 10 μm, −20°C; sections were thaw-mounted onto slides and air dried for 1–3 h. Sections were mounted in Vectashield (Vector Laboratories, Burlingame, CA), coverslipped, and examined with a Leitz Diaplan microscope with epi-illumination using filter block A (ultraviolet excitation at 340- to 380-nm wavelength). Only cells containing FG and a visible nucleolus were counted as labeled. Counts were made at all serial sections cut across the main axis of the ganglion. Data are presented as the number of FG-labeled cells for each ganglion. Data on the cell diameters of FG-labeled cells are based on the means of the minimum and maximum axes for cells with a visible nucleolus.

Electrophysiology

**General procedures.** Experiments were performed on 71 male Sprague-Dawley rats (400–500 g). Food, but not water, was withheld for 24 h before surgery. The animals were anesthetized initially with an intraperitoneal injection of pentobarbital sodium (45–50 mg/kg) and subsequently maintained with a constant intravenous infusion of pentobarbital (5–10 mg·kg⁻¹·h⁻¹). The right femoral vein was cannulated for infusion of fluid and pentobarbital. The right femoral artery was cannulated and connected to a pressure transducer for monitoring blood pressure and heart rate. The mean arterial pressure was maintained at 80 mmHg with supplemental intravenous injection of 5% dextrose in saline administered in a bolus of 1–1.5 ml as required. The trachea was intubated to permit artificial ventilation with room air. The rat was paralyzed with pancuronium bromide (0.2–0.3 mg/kg iv) and mechanically ventilated with room air (~70 strokes/min, 2- to 2.5-ml stroke volume). Supplemental doses of pancuronium bromide (0.2–0.3 mg·kg⁻¹·h⁻¹) were given to maintain paralysis during the experiment. Core body temperature was maintained at 37°C by a hot water circulating heating pad placed under the rat and an overhead feedback-controlled heat lamp (thermoprobe inserted into the rectum; Yellow Springs Instrument, Yellow Springs, OH). At the end of experiments, rats were killed with an overdose of pentobarbital.

**Surgical procedures.** The abdomen was opened by a transverse epigastric incision 4–5 cm in length. The left greater splanchnic nerve was isolated from the surrounding fatty tissues, and a pair of Teflon-coated, 40-gauge stainless steel wires stripped at the tips were placed around the nerve and sealed with hydrophilic vinyl polysiloxane (Reprosil, Dentsply International, Milford, DE).

The stomach was intubated with flexible Tygon tubing (2.3-mm OD, 1.3-mm ID) and Intramedic tubing (PE50; 0.965-mm OD, 0.58-mm ID) via the mouth, esophagus, and cardia. The catheter was secured by a ligature around the esophageal-gastric junction, with care being taken not to damage the vagus nerve. The blood supply and nerves innervating the stomach remained intact. Another Tygon tube (4.0-mm OD, 2.4-mm ID) was introduced distally through the pylorus and secured by a ligature placed caudal to the pyloric sphincter; the duodenum was ligated close to the pyloric ring. For gastric distension (GD), the distal catheter was connected to a pressurized reservoir containing saline. The abdomen was closed with silk sutures.

The thoracolumbar spinal cord was exposed by laminectomy (T7–T11), and the rat was suspended from thoracic and lumbar spinous processes. The splanchnic nerve was isolated from the surrounding fatty tissues, and a pair of Teflon-coated, 40-gauge stainless steel wires stripped at the tips were placed around the nerve and sealed with hydrophilic vinyl polysiloxane (Reprosil, Dentsply International, Milford, DE).

**Recording of afferent nerve action potentials.** Recordings were made from the distal cut end of the central processes of primary afferent fibers. A length of nerve fiber was draped over a black microscope plate immersed in warm (37°C) mineral oil. The dorsal rootlet was split into thin bundles, and fine filaments were teased from the bundle to obtain a single unit. Electrical activity of the single unit was recorded by placing the fiber over one arm of a bipolar silver-silver chloride electrode. A fine strand of connective tissue was placed over the other pole of the electrode for differential recording. Action potentials were monitored continuously by analog delay and displayed on a storage oscilloscope after low-noise AC differential amplification. Action potentials were processed through a window discriminator and counted (1-s bin width) on-line using the spike2/CEP 1401 data acquisition program (Cambridge Electronic Design, Cambridge, UK). Peristimulus time histograms, intragastric pressure, intragastric temperature, and blood pressure were displayed on line continuously. Data were also recorded on tape for later analysis.

**Experimental Protocol**

Splanchnic nerve input to the T9–T10 dorsal root was identified first by electrical stimulation of the left greater splanchnic nerve (a single 0.5-ms square-wave pulse at 1–15 V). Single fibers were classified based on the onset latency of conduction velocity (CV) combined by estimating with a piece of thread the distance between stimulation and recording sites postmortem and dividing the conduction distance by conduction time (time between stimulus artifact and evoked response). Fibers with a CV < 2.5 m/s were considered unmyelinated C-fibers, and those with a CV > 2.5–25.0 m/s were considered thinly myelinated Aδ-fibers. The isolated stomach was connected to a pressurized fluid reservoir through the pyloric catheter. The fluid reservoir was connected to a distension control device (22, 69); intragastric pressure was monitored via an in-line low-volume pressure transducer. At rest, saline

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analyzed using Student’s t-test or ANOVA. A value of $P < 0.05$ was considered statistically significant.

**Chemicals and Drugs**

$D$-Glucose, glycerol, hydrochloric acid, and sodium hydroxide were purchased from Fisher Scientific (Fair Lawn, NJ); $L$-arginine and potassium oleate were purchased from Sigma (St. Louis, MO) and dissolved in saline. Histamine hydrochloride (mol wt 184.1), serotonin hydrochloride (mol wt 212.7), PGE$_2$ (mol wt 352.5), and bradykinin (mol wt 1,060.2) were purchased from Sigma and dissolved in saline.

**RESULTS**

**Retrograde Labeling**

FG injected into the stomach labeled cells bilaterally in DRG. There were similar numbers of cells on each side. Labeled cells were distributed in DRGs T$_4$–L$_2$ with peak distribution at T$_9$ or T$_{10}$ (Fig. 1B). The distributions in DRG of labeled neurons after FG injections into the fundus, corpus, and pyloric antrum were similar. FG-labeled cells ranged in size from 17 to 47 $\mu$m in diameter, most being in the 30- to 40-$\mu$m range (mean diameter $35.3 \pm 0.2$ $\mu$m; Fig. 1C). The distribution of cell diameters was unimodal. Relatively few cells were smaller than 30 $\mu$m or larger than 40 $\mu$m.

**GASTRIC SPLANCHNIC AFFERENTS**

![Photomicrograph of a retrogradely labeled cell containing fluorogold (FG) and a visible nucleolus in the T$_9$ dorsal root ganglia.](http://ajpgi.physiology.org/)
μm. Labeling in the DRG was distributed throughout the ganglion; no localized distribution was observed.

**Fiber Sample**

A total of 997 afferent fibers were identified by electrical stimulation of the left greater splanchnic nerve. Fifty-five percent (n = 544) of the fibers had C-fiber (mean CV = 1.2 ± 0.02 m/s; range 0.4–2.4 m/s), and forty-five percent (n = 451) had Aδ-fiber (mean CV = 7.8 ± 0.2 m/s; range 2.5–21.7 m/s) CVs; two fibers had faster CVs (32.6 and 40.7 m/s). Of 997 fibers, 31 (3%) responded to gastric distension; 6 (19%) were C-fibers (mean CV = 1.2 ± 0.3 m/s; range 0.7–2.3 m/s) and 25 (81%) were Aδ-fibers (mean CV = 7.6 ± 0.9 m/s; range 2.6–16.3 m/s). Twenty-eight fibers had some resting activity; three fibers were not spontaneously active. The resting activity of 25 fibers was ≤1 imp/s (mean = 0.5 ± 0.1 imp/s, range: 0.01–2.9 imp/s; n = 28).

**Response to GD**

Responses to phasic GD typically (30 of 31 fibers) exhibited an initial dynamic response followed by a slowly adapting response during maintained GD. Slow adaptation to a tonic discharge was generally observed at all intensities of GD; an example is given in Fig. 2A. One fiber gave a nonadapting, sustained response during GD. After termination of GD, some fibers gave evidence of a period of poststimulus inhibition of spontaneous activity (Fig. 2B). The frequency of discharge fell below the resting level of activity after termination of GD in 9 of 31 fibers for a mean duration of 55.1 ± 14.9 s. Other fibers exhibited afterdischarge at rates greater than resting. Although we did not follow the duration of all afterdischarges, afterdischarges in 9 of 31 fibers continued for 4 s to >219 s after termination of 60-mmHg GD (see, e.g., Fig. 2C). No fibers gave on-off type, rapidly adapting responses. The characteristics of gastric splanchnic afferent fibers are summarized in Table 1, in which they are contrasted with a group of gastric vagal afferent fibers studied under similar conditions (50).

**Reproducibility of Responses**

Six fibers were tested for response to repetitive GD at 60 mmHg for 30 s. Four of the six fibers studied showed modest adaptation to repeated GD. Overall, the mean response of the six fibers after the 10th distension was 79.8 ± 9.0% of the response to the 1st distension (paired t-test, P < 0.05). Although not statistically significant, there is a clear indication that response magnitude to GD decreases at this interstimulus interval. Figure 3 shows examples of responses to 10 successive gastric distensions at 4-min intervals.

**Table 1. Characteristics of afferent fiber responses to 60-mmHg gastric distension**

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Splanchnic</th>
<th>Vagus*</th>
</tr>
</thead>
<tbody>
<tr>
<td>n (sample)</td>
<td>24</td>
<td>7</td>
</tr>
<tr>
<td>Response threshold</td>
<td>Low</td>
<td>High</td>
</tr>
<tr>
<td>mmHg</td>
<td>1.4 ± 1.5</td>
<td>≥30</td>
</tr>
<tr>
<td>Spontaneous activity, imp/s</td>
<td>0.4 ± 0.1</td>
<td>0.4 ± 0.2</td>
</tr>
<tr>
<td>Adaptation (n)</td>
<td>Slow</td>
<td>Non</td>
</tr>
<tr>
<td></td>
<td>24</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0</td>
</tr>
<tr>
<td>Post-GD inhibition, % of sample (n)</td>
<td>25(6)</td>
<td>43(3)</td>
</tr>
<tr>
<td>duration (s)</td>
<td>56.3 ± 17.3</td>
<td>52.7 ± 34.1</td>
</tr>
<tr>
<td>Post-GD after discharge, % of sample (n)</td>
<td>29(7)</td>
<td>33(3)</td>
</tr>
<tr>
<td>duration (s)</td>
<td>4 ± &gt;219</td>
<td>22,201</td>
</tr>
<tr>
<td>Maximum response to 60 mmHg, imp/s</td>
<td>14.8 ± 1.7</td>
<td>9.6 ± 1.7</td>
</tr>
<tr>
<td>Latency to maximum response, s</td>
<td>10.3 ± 0.8</td>
<td>13.7 ± 3.2</td>
</tr>
</tbody>
</table>

Data are means ± SE; n, no. of fibers. GD, gastric distension; imp, impulse. *Data from Ref. 50.
Stimulus-Response Functions

Responses to graded GD were studied in all 31 fibers. Fibers generally gave monotonic increases in firing with increasing distending pressure (5 to 60 or 80 mmHg). Extrapolation of the linear portion of individual SRFs revealed that gastric splanchnic nerve afferent fibers exhibit a wide range of thresholds for response to GD (0–53 mmHg). We identified 24 fibers as having low thresholds for response. Most (13) responded to the lowest intensity of GD tested (5 mmHg), and 23 of 24 responded to 10-mmHg GD. The extrapolated mean response threshold for these 24 fibers was 1.4 ± 1.5 mmHg. Seven of the thirty-one fibers only responded to distending pressures ≥30 mmHg and were thus classed as high threshold (HT). Consistent with previous findings in the rat pelvic nerve (62, 63), low-threshold (LT) gastric splanchnic nerve afferent fibers encoded distending stimuli throughout the range of distending pressures tested and gave, as a group, greater-magnitude responses to all pressures tested. Examples are illustrated in Fig. 4, and data are summarized in Fig. 5.

Chemo- and Thermosensitivity

The effects of nutrients, irritants, and/or thermal stimuli were tested on 18 of the 31 mechanosensitive fibers. Fifteen of the fibers had low thresholds for response to GD (11 Aδ-fibers and 4 C-fibers), and three fibers (2 Aδ and 1 C) had a high threshold for response to GD.

Chemosensitivity. The effect of instillation of 20% glucose (3 tests), 300 mM L-arginine (2 tests), or 40 mM potassium oleate (1 test) into the stomach on spontaneous activity and responses to graded GD (5–80 mmHg) was tested on three mechanosensitive splanchnic nerve afferent fibers. Neither spontaneous activity nor mechanosensitivity in this small sample of fibers was affected.

The effect of instillation of 12% glycerol into the stomach on spontaneous activity and responses to graded GD (5–80 mmHg) was tested on two fibers. Glycerol remained in the stomach for the duration of the experiment. The mean resting activity of one fiber increased slightly (from 0.3 to 0.5 imp/s) after intra-
gastric instillation of glycerol. Both fibers, however, were sensitized, as evidenced by increased magnitudes of response to graded GD 15, 60, and 120 min after intragastric instillation of glycerol. In the example given in Fig. 6A, response magnitude increased progressively over time.

The effect of IS on spontaneous activity and responses to graded GD was tested on four fibers. IS remained in the stomach for the duration of the experiments. The spontaneous activities of fibers were unaffected after intragastric instillation of IS, but two of four fibers exhibited sensitization of responses to graded GD after intragastric instillation of IS. Response magnitude to GD was increased and response threshold decreased after IS treatment (see example in Fig. 6B).

**Thermosensitivity.** The effect of intragastric perfusion of hot and/or cold saline was tested. Cold was tested first, and none of eight fibers responded. Four fibers were also tested for responses to a second cold stimulus 10 min after the first cold stimulus; none of these fibers responded on the second trial. The responses of five fibers to graded intensities of GD were tested 10 min after cold stimulation. The fibers showed moderate desensitization or no change after cold stimulation.

Four of twelve mechanosensitive afferent fibers responded to gastric instillation of heated saline. Spontaneous activity in these four fibers increased from a mean of 0.3 ± 0.1 imp/s to a mean maximum of 8.0 ± 4.4 imp/s during intragastric perfusion of heated saline; the estimated mean response threshold was 47.2 ± 1.8°C (n = 4; range 44–52°C), which, because the thermistor monitored intraluminal temperature, is likely greater than the response threshold at the receptive ending. An example of response to heat is given in Fig. 7A. Two fibers were also tested for possible sensitization of response to a second heat stimulus 10 min after the first stimulus. In neither instance was the response to the second heat stimulus different from the response to the first heat stimulus.

The responses of 10 fibers (4 heat sensitive and 6 heat insensitive) to graded intensities of GD were tested 10 min after heat stimulation. Only heat-sensitive (2 of 4) fibers exhibited sensitization to GD after heat stimulation. Figure 7B shows sensitization of responses of a fiber to GD 10 min after heat stimulation. The six heat-insensitive fibers showed moderate desensitization or no change in response to GD after heat stimulation.
DISCUSSION

The present study found that gastric splanchnic nerve afferent fibers innervating the stomach of the rat exhibit a range of response thresholds to constant-pressure GD. A proportion of the fibers studied responded only to distending pressures $\geq 30$ mmHg, an intensity we consider to be in the noxious range, suggesting that acute gastric pain is likely signaled by activation of these fibers. In support of this suggestion, visceromotor responses to GD in unanesthetized rats are apparent at distending pressures $\geq 30$ mmHg (48). In contrast, a previous study of gastric vagal afferent fibers revealed no HT fibers in that sample (50). Together, these studies support clinical evidence that acute gastric pain is conveyed to the central nervous system by gastric splanchnic afferent fibers. Furthermore, exposure of the luminal surface of the stomach to irritant chemicals or prior heat stimulation led to sensitization of responses to gastric distension. Accordingly, gastric splanchnic afferent fibers have the ability to sensitize and thus contribute to altered sensations from the stomach that characterize disorders such as functional dyspepsia.

Gastric Innervation

Although the splanchnic nerve innervation of the stomach has been examined previously, we anticipated a low yield of mechanosensitive fibers in the electrophysiological experiments and wanted to target in rats from our vendor the most appropriate dorsal roots for study. Retrograde tracing studies (8, 15, 23, 24, 33, 46, 67) document that most splanchnic nerve afferent fibers from the stomach enter the spinal cord through the thoracolumbar (T3–L3) dorsal roots, but there is apparent variation between and among strains of rats. For example, in an unspecified strain of albino rat, cells retrogradely labeled from the stomach were observed bilaterally in spinal ganglia T4–L1, being most numerous in ganglia T8–T10 (46), whereas in male Sprague-Dawley rats labeled cells were found in DRG bilaterally from T6 to L1, with the greatest numbers in T11 (23, 24). Similarly, minor differences have been reported for the pelvic nerve innervation in two strains of rats (57). The present results in male Sprague-Dawley rats, revealing the largest input from the stomach in the T9 and T10 dorsal roots, are largely consistent with the horseradish peroxidase studies of Neuhuber and Niederle (46) and differ slightly from results in male Sprague-Dawley rats reported by others (23, 24, 67). Similarly, the cell body diameter determined in the present study (principally 30–40 $\mu$m) is consistent with other reports.

We injected tracer into restricted areas of the stomach to determine whether the splanchnic nerve innervation of the rat stomach might be organized viscero-
topically. We found that the afferent innervation of the fundus, corpus, and pyloric antrum was similar in distribution in thoracolumbar DRG. Moreover, labeled cells from all stomach areas were similarly most numerous in ganglia T₈–T₁₀.

Response Thresholds

Recent studies provide evidence for the existence of separate populations of LT and HT mechanosensitive afferent fibers in the viscera (e.g., esophagus, gallbladder, colon, urinary bladder, ureter, uterus; for review, see Ref. 65). Cervero (10) first described two populations of afferent fibers in the splanchnic nerve innervating the gallbladder of the ferret. Most of the fibers responded to low intensities (2–5 mmHg) of gallbladder distension, but about one-third responded to distending pressures >20 mmHg, which was interpreted as evidence for their role in nociception. In the splanchnic nerves supplying the esophagus of the opossum (60, 61), about two-thirds of the fibers were LT and one-third had high thresholds for response. Pan and Longhurst (55) described LT and HT splanchnic nerve C-fibers innervating the gastrointestinal tract of the cat. The proportion of HT fibers typically reported is 20–30% of the sample studied and was 22% (7 of 31) of the sample reported here. Given their high thresholds for response (≥30 mmHg), these fibers are likely important to acute stomach pain (i.e., are nociceptors). LT fibers, on the other hand, likely mediate events that are not sensed or play a role in nonpainful sensations that arise from the stomach.

Receptor Location

In a previous study of gastric vagal afferent fibers (50), we identified receptive fields of fibers. We were unable to similarly determine fiber receptive fields in the present study. Rats were suspended from thoracic and lumbar spinal clamps to stabilize the preparation for recording from short-length T₉ and T₁₀ dorsal roots. Recordings were disrupted and fibers lost when we attempted to access and probe the stomach surfaces to search for receptive fields. Accordingly, we cannot be certain that HT fibers encountered here did not have mucosal or distant (e.g., serosal or mesenteric) receptive fields. For several reasons, we believe that receptive fields of the HT fibers studied here were in the muscle layers.

Responses of splanchnic nerve afferent fibers innervating the stomach to controlled, phasic GD were characteristically dynamic and slowly adapting. Similar slowly adapting responses to sustained GD have been reported for gastric vagal afferent fibers (3, 6, 17, 18, 25, 35, 36, 40, 47, 50–54, 72, 73) and for visceral nerves innervating other hollow organs (see Refs. 64 and 65 for review). Such responses typically encode distending pressure; are commonly associated with receptors variously termed stretch, in-series, or tension receptors; and are located in hollow organ muscle (see Refs. 11 and 64 for review). Gastric mucosal afferent fibers, in contrast, are rapidly adapting to a mechanical stimulus, giving an on-off response when a steady mechanical stimulus is applied and terminated but no response when the stimulus is maintained (41). Similarly, on-off responses have been described for gastric serosal receptors (4, 14) and in the gastrointestinal tract and pacinian corpuscles in the peritoneum (43, 58). No fibers in the present study gave on-off, rapidly adapting responses suggestive of sensitivity to movement of fluid across the mucosa.

Erroneously high thresholds for response could arise when distending at a site distant from the receptor in the viscus. For example, using two balloons placed in the cat colon, Jänig and Koltzenburg (39) demonstrated that thresholds for response to distension are high when the balloon distended is distant from the receptive ending. It is unlikely in the present study that we would have preferentially excited one and not all branches of an afferent fiber because we employed fluid, not balloon, distension that completely filled the nonspherical stomach. Rapid fluid filling of the stomach, however, could have indirectly activated distant serosal or mesenteric mechanosensitive receptors by increasing tension in stomach muscle and/or distorting the mesentery. Although mesenteric serosal afferent fibers can be slowly adapting in response to distension (20), changes in mesenteric tension during distension do not closely correlate with the magnitude of distension (20, 58). Other investigators describe serosal afferent fibers as rapidly adapting in response to intestinal distension or localized pressure on receptive spots and desensitizing to repetitive stimulation (4). In a recent study of colonic mucosal, serosal, and muscle receptive fields (43), neither mucosal nor serosal afferent fibers responded to stretch; only afferent fibers with endings in muscle gave sustained, slowly adapting responses to stretch. Finally, luminal challenge with thermal and chemical stimuli led to sensitization of responses to distension, further arguing against a distant site of activation. Accordingly, despite our inability to locate the receptive spots/fields of the fibers studied here, we believe that the receptive endings were in stomach muscle.

Sensitization

Sensitization of responses to gastric distension was apparent after intragastric instillation of chemical irritants (glycerol or IS) or heated saline. Six of ten fibers exposed to these stimuli showed sensitization to subsequent gastric distension. Glycerol is an irritant that promotes a bowel movement when infused rectally (59) and induces high-amplitude colonic contractions in humans (31). In animals, intracolonic instillation of glycerol stimulates colonic motility and induces contractions (74). Glycerol sensitized responses to GD of both fibers tested; spontaneous activity was not appreciably changed, but response magnitude increased. Given its irritant nature, glycerol may induce contractions in the stomach or alter compliance of the organ (which we did not monitor). Because spontaneous activity was not affected, however, and response magnitudes progres-
sively increased over time to high-intensity distension, it seems unlikely that a decrease in gastric compliance would explain the outcome observed.

Intragastric instillation of IS increased the magnitude of response of two of four fibers to GD throughout the range of distending pressures tested and also reduced the thresholds for response of both fibers. We showed previously (69) that this cocktail of inflammatory mediators, developed by Handwerker and Reeh (30) to mimic the key mediators released or synthesized at the site of an acute somatic inflammation, sensitizes pelvic nerve afferent fibers to colonic distension. Luminal application of IS is not associated with obvious macroscopic damage to the stomach and thus represents a relatively mild insult.

A number of studies have reported that mechanosensitive visceral afferent fibers are responsive to and/or sensitized by chemicals, principally algesic or irritant chemical. It has long been known that mechanosensitive gastric mucosal afferent fibers in the vagus nerve are often also sensitive to chemical stimuli (37). Others have since documented that mechanosensitive afferent fibers innervating colon or urinary bladder muscle also respond and/or sensitize to chemical stimuli (e.g., bradykinin, capsaicin, acetic acid, mustard oil, xylene, IS; Refs. 19, 27, 32, 38, 43, 62, 63, 66, 69–71). In the upper gastrointestinal tract, Longhurst extensively studied the chemosensitivity (serotonin, histamine, bradykinin) of afferent fibers innervating stomach, proximal small intestine, and mesentery in the cat, many of which are also mechanosensitive (e.g., Refs. 21, 26, 56; see Ref. 42 for overview). Adelson et al. (1, 2), employing an in vitro preparation of rat mesenteric nerve, reported H$_2$O$_2$- and bradykinin-sensitive splanchnic afferent C-fiber units. Recently, Brunsden and Grundy (9) examined the effects of inflammatory mediators on rat jejunal afferent fibers in vitro, reporting that afferent discharges induced by bradykinin were augmented by histamine, adenosine, and PGE$_2$. The IS employed here is a cocktail of similar inflammatory mediators.

Because mechanosensitive gastrointestinal afferents sensitize after insult or even after a noninjurious stimulus, they can contribute to central hyperexcitability and to visceral hyperalgesia. There are a number of clinical conditions, categorized as functional bowel disorders (e.g., nonulcer or functional dyspepsia), that are characterized by discomfort and pain in the absence of tissue inflammation or apparent pathology (44, 45). These disorders are complex and involve both peripheral and central contributions. It is apparent that a significant component of the discomfort and pain associated with such functional bowel disorders is associated with altered sensory input and/or altered integration in the central nervous system. Improved knowledge of adequate stimuli for receptors in the gastrointestinal tract and their basic physiology will clarify the extent to which the peripheral sensory component contributes to the altered sensations and pain that characterize functional bowel disorders.

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